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TITLE: DEVELOPMENT OF ANTIBIOTIC FORMULATIONS FOR COMBAT CASUALTY CARE

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The object of this contract was to develop biodegradable, controlled-release formulations for the local administration of cefamandole and tobramycin. These formulations were designed to prevent infection of wounds resulting from warfare. Under the original scope of the research program we planned to develop two dosage forms for each drug -- microspheres and microbeads. The microsphere formulations, antibiotic spheres less than 1 mm in diameter, were designed for topical administration. The microbead formulations, antibiotic spheres about 5 mm in diameter, were designed for intraosteal administration. On October 18, 1993, the contract for this research program was modified to delete the tobramycin microbead deliverable from the contract requirements.

To meet the objective of this research program, we prepared and characterized microsphere and microbead formulations with cefamandole and tobramycin. We developed a cefamandole microsphere formulation that released cefamandole for 28 days *in vitro* and a cefamandole microbead formulation that released cefamandole for 35 days *in vitro*. And we developed a tobramycin microsphere formulation that released tobramycin for 35 days *in vitro*. Samples of these formulations were sent to the U.S. Army Institute of Dental Research (USAIDR) for in-house evaluation (*in vivo* efficacy).

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DEVELOPMENT OF ANTIBIOTIC FORMULATIONS FOR COMBAT CASUALTY CARE

I. INTRODUCTION

This document is our Final Report on Contract DAMD17-92-C-2014. It covers research performed during the period from July 16, 1992, to February 21, 1994.

The objective of this research program was to develop biodegradable, controlled-release formulations for the administration of cefamandole and tobramycin. These formulations were designed to prevent infection of wounds resulting from warfare. Under the original scope of the research program we planned to develop two dosage forms for each drug -- microspheres and microbeads. The microsphere formulations, consisting of particles less than 1 mm in diameter, were designed for topical administration. The microbead formulations, consisting of particles about 5 mm in diameter, were designed for intraosteal administration. Once administered directly to a wound, both the microspheres and microbeads were designed to release an initial burst of antibiotic to the wound. The remainder of the antibiotic is delivered at a relatively constant rate over a period of 28 to 42 days. After administration, the excipient should completely degrade within 35 to 50 days.

Because the development of cefamandole and tobramycin microsphere formulations required a greater research effort than we had anticipated, we were unable to complete the development of both microbead formulations with the funds allocated for this research program. On October 18, 1993, the contract for this research program was modified to delete the tobramycin microbead deliverable from the contract.

To meet the objective of this research program, we prepared and characterized prototype microsphere and microbead formulations with each antibiotic, cefamandole and tobramycin. Samples of the best cefamandole and tobramycin microsphere formulations and the best cefamandole microbead formulation were sent to the U.S. Army Institute of Dental Research (USAIDR) for in-house evaluation (*in vivo* efficacy). More specifically, we prepared microsphere formulations comprising antibiotic (cefamandole or tobramycin) encapsulated within a poly(DL-lactide-co-glycolide) excipient (DL-PLG). We also prepared cefamandole microbeads from the cefamandole microspheres. Copies of the Sample Transfer Forms that accompanied each sample delivered to USAIDR are included in Appendix A.

II. DEVELOPMENT OF MICROSPHERE AND MICROBEAD FORMULATIONS

The development of an effective controlled-release delivery system is a complex process that requires the careful consideration of numerous parameters. The selection of the polymeric excipient and the microencapsulation process must be carefully made with respect to physiochemical properties of the drug and the release characteristics desired. The development of cefamandole and tobramycin delivery systems (microsphere and microbead formulations) involved the following tasks.

TASK 1: Select and obtain commercially available polymers.

TASK 2: Obtain cefamandole and tobramycin.

TASK 3: Establish analytical methods for cefamandole and tobramycin.

TASK 4: Determine the stability of the cefamandole and tobramycin in solution for *in vitro* release studies.

TASK 5: Determine the effect of gamma radiation on cefamandole and tobramycin.

TASK 6: Establish several characterization procedures for microsphere and microbead formulations.

TASK 7: Prepare and characterize microsphere formulations.

TASK 8: Prepare and characterize microbead formulations.

TASK 9: Send samples of microsphere formulations to USAIDR for evaluation.

TASK 10: Send samples of microbead formulations to USAIDR for evaluation.

A. Selection and Purchase of Polymers

We purchased 60:40 DL-PLG from Birmingham Polymer, Inc., for both the tobramycin and cefamandole formulations. During this contract, we used three lots of this 60:40 DL-PLG. The certificates of analysis for these polymers (BPI Lots 112-68-1, 112-88-1, and 112-95-1) are included in Appendix B of this report.

B. Selection and Purchase of Drugs

Cefamandole is available in several different forms. Cefamandole is sold as the free acid (cefamandole) and as the sodium salt (cefamandole sodium). In addition, it is sold as the formyl ester of cefamandole (cefamandole nafate); this form contains about 5 wt % sodium carbonate.

Early on, we evaluated two forms of cefamandole (cefamandole sodium and cefamandole nafate), both purchased from Sigma Chemical Company (St. Louis, MO). We were unable to prepare microspheres that release as long as required *in vitro* (28- to 35-day duration) with either of these forms of drug. Later we ordered 500 g each of cefamandole and cefamandole nafate from Interchem Corporation (Paramus, NJ). In February 1993, we received the cefamandole and cefamandole nafate from Interchem Corporation. Certificates of Analysis for these antibiotics are included in Appendix C of this report. (Note: In this report, all references to the drug content of microspheres of microbeads or to quantities of cefamandole released from these formulations are stated in terms of the free acid form of cefamandole.)

Tobramycin is available as the free base (tobramycin) and as the sulfate salt (tobramycin sulfate). Initially we obtained small quantities (about 5 g) from ICC Industries (New York, NY), of both tobramycin and tobramycin sulfate. With these small quantities of drug, we were unable to prepare microspheres that release as long as required *in vitro* (28- to 35-day duration) with either form of the drug. Later we ordered 500 g of tobramycin and 500 g of tobramycin sulfate from ICC Industries. We received both drug forms in late January 1993. (Note: In this report, all references to the drug content of microspheres of microbeads or to quantities of tobramycin released from these formulations are stated in terms of the free base form of tobramycin.)

C. Development of Analytical Methods

Initially, we assayed cefamandole and cefamandole nafate by HPLC. We modified a published HPLC method[1] to optimize it for our equipment and application. A summary of our method follows:

Instrument:	Hewlett-Packard HP-1090 HPLC
Column:	Hewlett-Packard Hypersil® ODS, 3 μm, 60 x 4.6 mm
Mobile Phase:	83.5 vol % 0.1 M sodium phosphate (pH 6.0)
	16.5 vol % acetonitrile
Flow:	2.2 mL/min
Wavelength:	254 nm
Injection Vol.:	100 μL
Linear Range:	0.004-1.200 mg of cefamandole/mL
Limit of Detection:	0.002 mg of cefamandole/mL

Later we determined the cefamandole and cefamandole nafate for core loadings and *in vitro* release kinetics by UV spectrophotometry rather that by HPLC. Because our

samples did not contain any biological material or other impurities that absorb at 254 nm, we did not need to use an HPLC method to assay our *in vitro* release samples.

To quantify tobramycin and tobramycin sulfate, we used a colorimetric assay based on the Hantzsch Reaction.[2,3] A dihydrolutidine derivative is formed when the primary amino groups present in aminoglycoside antibiotics undergo condensation with acetylacetone and formaldehyde under acidic conditions (pH 2.6). After derivatizing the tobramycin, samples are assayed spectrophotometrically by measuring the absorbance at 356 nm. Derivatized tobramycin and tobramycin sulfate adhere to Beer's Law and can be quantified by this method. The limit of detection for tobramycin in this assay is 1.3 μg/mL. The linear range is 1.3 μg/mL to 100 μg/mL.

D. Evaluation of Solution Stability of Cefamandole and Tobramycin for In Vitro Release Studies

The solution stability of the antibiotic is important because it affects the reliability of *in vitro* release studies. That is, if released antibiotic degrades before a sample is taken, the amount of antibiotic released is lower than actual.

We performed solution-stability studies in buffer (0.01 M sodium phosphate, pH 7.4) at 4 °C and at 37 °C with both cefamandole and tobramycin. At 37 °C, we observed a greater than 10% loss for both cefamandole and cefamandole nafate. Such losses prevent the use of a direct *in vitro* release method. Therefore, we used an indirect *in vitro* release method for cefamandole and cefamandole nafate formulations.

We found that tobramycin was stable for at least 12 days at both conditions (37 °C, 4 °C, stored with and without 60:40 DL-PLG). The results obtained from the tobramycin sulfate were not as conclusive. But, there was not a significant stability problem for either tobramycin or tobramycin sulfate.

E. Effect of Gamma Radiation on Cefamandole and Tobramycin

Typically, controlled-release antibiotic formulations are terminally sterilized by gamma irradiation. Therefore, it is important to know how exposure to gamma radiation will affect all forms of cefamandole and tobramycin. We exposed samples of each drug to 0.5, 1.0, 2.0, and 2.5 Mrad of gamma radiation. We assayed the drug samples before and after exposure to gamma irradiation to determine if the gamma radiation affected the quantification of cefamandole or tobramycin in our analytical procedures. We did not evaluate the effect of the gamma radiation on the biological activity of the drugs.

The results of the initial evaluation of cefamandole were not conclusive. We have, however, determined from more recent *in vitro* release studies that sterilization by gamma irradiation does not adversely affect the release of cefamandole from the microspheres.

In the initial study, there were some inconsistencies in the irradiation data for the tobramycin sulfate, but subsequent analyses of tobramycin sulfate microspheres before and after sterilization indicated that the tobramycin sulfate was not adversely affected exposure to gamma radiation.

F. Establishment of Characterization Procedures For Microsphere and Microbead Formulations

We established procedures for evaluating the surface morphology of the microspheres by scanning electron microscopy (SEM), for determining the particle-size distribution of the microspheres, for determining the core loading (drug content) of cefamandole and tobramycin microspheres and microbeads, and for determining the *in vitro* release of drugs microspheres or microbead. These procedures are discussed in Section IV. of this report.

G. Preparation and Characterization of Microsphere Formulations

We prepared and characterized numerous microsphere formulations using either cefamandole, cefamandole sodium, or cefamandole nafate. And we prepared and characterized microsphere formulations from either tobramycin or tobramycin sulfate.

1. Cefamandole microspheres

Early in this research program, we prepared about 50 batches of microspheres using cefamandole sodium or cefamandole nafate, both purchased from Sigma Chemical Co. These batches were made using Southern's microencapsulation process. None of these 50 batches of microspheres released cefamandole for the desired duration of 28 to 35 days.

After we received cefamandole and cefamandole nafate from Interchem Corporation, we prepared more batches of microspheres with drugs from this source. These batches were made by Southern's microencapsulation process and by a phase-separation process. Again, the cefamandole nafate microspheres prepared by both processes released cefamandole too quickly (greater than 75% of the cefamandole released in 3 days). The cefamandole microspheres, however, released more slowly.

We chose the cefamandole batch with the best *in vitro* release characteristics as a model (Batch H474-050-01). The microspheres contained 7.3 wt % cefamandole and released only 60% of the cefamandole after 7 days. We scaled up the batch size for prototype Batch H474-050-01, then prepared, sterilized, and characterized samples to deliver to USAIDR for *in vivo* studies. To characterize the microspheres, we examined the surface morphology by SEM, determined of the size distribution of the microspheres, determined the core loading, and measured the release of cefamandole from the microspheres *in vitro*. Characterization data are given on Sample Transfer Form 7717-4 (Appendix A). The cefamandole microspheres (Composite H326-093-01S) had a core loading of 9.45 wt % cefamandole. The *in vitro* release studies showed about 85% of the cefamandole was released in 15 days, and the remaining drug was released by Day 28 of the study. Placebo

microspheres (Composite H326-098-01S) were prepared using the same process that was used to prepare the cefamandole microspheres. Both the cefamandole microspheres and the placebo microspheres were sterilized by gamma irradiation (2.5 Mrad ± 10%). The cefamandole microspheres and placebo microspheres were shipped to USAIDR on May 13, 1993.

2. *Tobramycin microspheres*

We prepared tobramycin and tobramycin sulfate microspheres using either modifications of Southern's microencapsulation process or a phase-separation process. We compared the *in vitro* release determinations from microsphere formulations with theoretical core loading of 2, 5, 10, and 15 wt% tobramycin. Generally, microsphere formulations prepared from tobramycin released drug too quickly (>90% released in 3 days). The tobramycin sulfate microsphere formulations released drug more slowly (30% to 85% released in four days). We chose the best tobramycin sulfate microsphere formulation (Batch H474-012-01) as a model to prepare samples for *in vivo* evaluation by USAIDR. Prototype microsphere Batch H474-012-01 was prepared by a modification of the phase separation process and contained 9.5 wt % tobramycin. In *in vitro* tests, only 60 wt % of the tobramycin was released from the microspheres after 15 days, and the microspheres continued to release tobramycin for 28 days.

We prepared 20 batches of tobramycin microspheres using the same process conditions used for prototype Batch H474-012-01. The 20 batches were combined to form Composite H474-125-01. The composite batch of microspheres was sterilized and characterized. Characterization of the microspheres included examination of surface morphology by SEM, determination of the size distribution of the microspheres, determination of core loading, and *in vitro* release of tobramycin from the microspheres. The tobramycin sulfate microspheres we prepared for delivery to USAIDR contained 9.1 wt % tobramycin. Complete characterization data are given on the Sample Transfer form STF 7717-1 (Appendix A-1). The *in vitro* release profile (Appendix A-3) shows that an initial release of about 25% of the tobramycin occurred within two days followed by a more gradual release of tobramycin over a period of 35 days. Placebo microspheres (Composite H474-149-01) were prepared using the same process used to prepare the tobramycin microspheres. Both the tobramycin sulfate microspheres and the placebo microspheres were sterilized by gamma irradiation (2.5 ± 10%). The tobramycin sulfate microspheres and placebo microspheres were shipped to USAIDR on May 13, 1993.

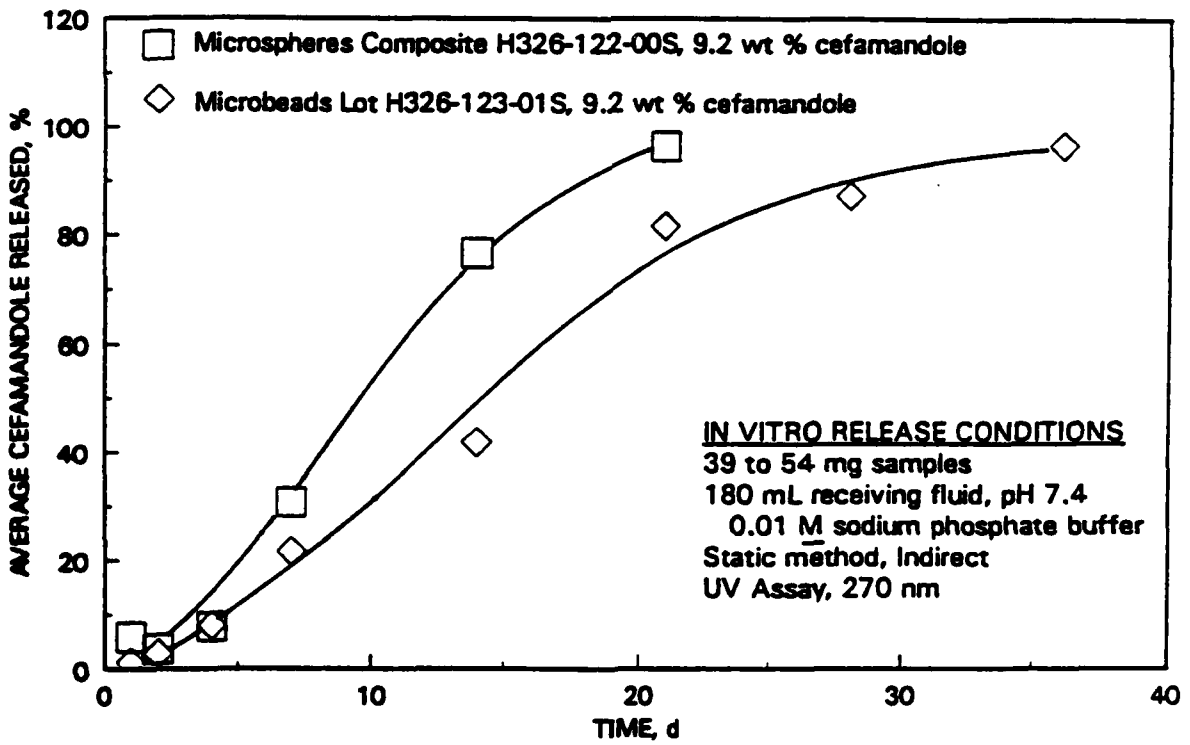
H. *Preparation and Characterization of Microbead Formulations*

1. *Cefamandole microbeads*

We used several methods to prepare cefamandole beads for preliminary evaluations. First, we prepared cores from cefamandole. These cores were coated with polymeric membranes to control the rate of release of cefamandole from the microbead. The polymeric coatings we applied were not adequate to extend the release of the antibiotic to greater than 28 days. In *in vitro* release studies, all of the drug leached out of the

microbeads in about three days. We tried several methods of coating the drug cores with a polymeric film, but none of polymer-coated drug cores provided the desired duration of release of drug for 28 days.

Having developed a cefamandole microsphere formulation that released drug for 28 days, we used the microencapsulated cefamandole to prepare microbeads that release cefamandole for the desired duration. We prepared additional batches of cefamandole microspheres like Composite H326-093-01S that was sent to USAIDR for evaluation. We used a tablet press equipped with a cylindrical die and two nearly hemispherical punches (Elizabeth Carbide Die Co., Inc., McKeesport, PA) to prepare microbeads from cefamandole microspheres. Each microbead has dimensions of approximately 3.9 mm x 4.4 mm (± 5%) and has a mass of 45 mg ± 10%. Characterization of the microbeads included examination of the surface morphology by SEM, determination of core loading, and determination of *in vitro* release. In *in vitro* release studies, the microbeads remained intact up to about 21 days. At this point the beads began to fall apart due to polymer degradation but enough polymer remained to continue to control the release of the cefamandole. We determined the *in vitro* release of cefamandole from the microspheres as well as from the microbeads. As shown below, the release of cefamandole from the microbeads was extended from 21 to 35 days.



In Vitro Release Profiles for
Cefamandole Microspheres and Microbeads

These results along with the results of our examination of the surface and interior sections of the microbeads by SEM (See Appendix A-17) confirm that the microspheres remained intact when molded into beads. Complete characterization data for the microbeads are presented on Sample Transfer Forms STF 7717-7 (Appendix A). Like the cefamandole microbeads, placebo microbeads (Lot H326-127-01) were prepared from placebo microspheres (Composite H326-126-01). The cefamandole microbeads and placebo microbeads were sterilized by gamma irradiation (2.5 ± 10%). The Cefamandole microbeads and placebo microbeads were sent to USAIDR on February 10, 1994.

2. *Tobramycin microbeads*

We also prepared cores for microbeads from tobramycin sulfate. We coated some of these cores with a polymeric membrane to control the rate of release of tobramycin from the microbead. The uncoated tobramycin microbeads completely dissolved in water in about an hour. The polymeric coatings we applied were not adequate to extend the release of the antibiotic to greater than 28 days. All of the drug leached out of the microbeads in about three days. We were unable to complete the development of the tobramycin microbead formulation with funds allocated for this research program. The contract was modified (effective October 23, 1993) to delete this Deliverable from the contract.

I. Delivery of Microsphere and Microbead Samples to USAIDR

We completed the preparation of Deliverable Set 1 (tobramycin microspheres) and Deliverable Set 2 (cefamandole microspheres) and shipped these samples to USAIDR on May 13, 1993. We completed the preparation and characterization of Deliverables Set 3 (cefamandole microbeads) and shipped these samples to USAIDR on February 10, 1994. Deliverables Set 4 (tobramycin microbeads) was deleted from the contract requirements. The components of Deliverables Set 1, Set 2, and Set 3 are given below.

Copies of the Sample Transfer Forms that accompanied the samples are in Appendix A. Characterization data for these samples are shown on the Sample Transfer Forms.

On February 10, 1994, we also sent to USAIDR the remainder of the antibiotics purchased with funds from this contract and requested equipment (HPLC column).

MICROSPHERE AND MICROBEAD SAMPLES DELIVERED TO USAIDR

Deliverable	STF 7717	Amount delivered, g	Core loading, wt % drug		Mean particle size, μm
			Theoretical	Actual	
Set 1					
Tobramycin Microspheres	-1R1	50 ± 1	15	9.1	357
Placebo microspheres	-2	25 ± 1	0	0	338
Tobramycin (free powder)	-3	25 ± 1	--	--	--
Set 2					
Cefamandole microspheres	-4R1	50 ± 1	15	9.5	357
Placebo microspheres	-5R1	25 ± 1	0	0	338
Cefamandole (free powder)	-6	25 ± 1	--	--	--
Set 3					
Cefamandole microbeads*	-7	50 ± 1	15	9.2	--
Placebo microbeads*	-8	50 ± 1	0	0	--
Cefamandole (free powder)	-9	359 ± 1	--	--	--

* Microbead size = 3.4 mm x 4.4 mm ± 5 %

III. CHARACTERIZATION PROCEDURES

A. Cefamandole

1. Extraction procedure for core-loading determinations

The extraction procedure for determining the core loadings (drug content) of cefamandole and cefamandole nafate microspheres and microbeads is described below. Core-loading determinations were routinely done in triplicate. (Note: All core loadings are expressed in terms of cefamandole.)

> Weigh approximately 40 to 50 mg of microspheres or microbeads. Transfer them to a 50-mL volumetric flask and add 30 to 40 mL of methylene chloride. Let the microspheres or microbeads stand in the methylene chloride for at least 1 hour. The methylene chloride will dissolve the polymeric excipient. Dilute the sample to the mark with methylene chloride. Then, an aliquot (about 3 mL) is removed from each flask and placed in a quartz cuvette. The samples are then assayed spectrophotometrically by measuring the absorbance of each sample at 270 nm.

We then calculate the core loading by the equation listed in Section IV.C.1. of this report.

2. In vitro release procedure

To determine the *in vitro* release characteristics for cefamandole or cefamandole nafate from microspheres or microbeads, we use an indirect *in vitro* method. This method works well for compounds, such as cefamandole and cefamandole nafate, which have stability problems in receiving fluid. This procedure is described below. *In vitro* release determinations are routinely done in triplicate. (Note: All *in vitro* release results are expressed in terms of cefamandole.)

> Weigh out approximately 40 to 50 mg of microspheres or microbeads into a 5 cm x 5 cm nylon (30 μm) pouch and heat seal the pouch. Place the pouch in an 250-mL narrow-mouth bottle. Repeat for each time point to be analyzed. Add 180 mL of receiving fluid (0.01 M sodium phosphate buffer, pH 7.4) and place the sample in an incubator kept at 37 °C. At the appropriate time, remove a sample and draw off the receiving fluid. When the microspheres or microbeads are dry, perform a core-loading determination as described in Section IV.A.1. above to quantify the drug remaining in the microspheres or microbead.

To calculate the amount of cefamandole released *in vitro*, use the equation listed in Section V.C.3. of this report.

B. Tobramycin

To characterize the tobramycin and tobramycin sulfate microspheres, we examined the surface morphology by scanning electron microscopy (SEM), determined the average core loading of the microspheres, and determined the *in vitro* release profile of the microspheres. Procedures for the core-loading determination and for the *in vitro* release determination are given below.

1. Core-loading determination

Weigh out approximately 40 to 50 mg of microspheres or microbeads and transfer them to a scintillation vial. Add 5 mL of 1 N sodium hydroxide to the vial. Be certain that all of the microspheres are covered with sodium hydroxide. Let stand overnight. After the microspheres or microbeads have dissolved, neutralize the sample with 1 N hydrochloric acid. Transfer the sample to a 25-mL volumetric flask and dilute to the mark with Nanopure® water. The sample is then assayed using the colorimetric assay based on the Hantzsch reaction. Note: a control sample that contains only the antibiotic is also run with each core-loading determination.

2. In vitro release procedure

Weigh out approximately 40 to 50 mg of tobramycin or tobramycin sulfate microspheres or microbeads, add 10 mL of receiving fluid (Nanopure water) and store at 37 °C. Periodically, the receiving fluid is removed for analysis and replaced with fresh receiving fluid. Typically samples are collected at Hours 1 and 6; Days 1, 2, 3, 7, 14, 28, 35. Samples are assayed using the colorimetric assay based on the Hantzsch reaction.

C. *Calculations for Characterizition Methods*

1. *Calculations for core loading*

$$\text{Core loading wt \% antibiotic} = \frac{\text{(Antibiotic assayed, mg/mL)(Dilution factor, mL)}}{\text{(Sample wt. mg)}} \times 100\%$$

2. *Calculations for antibiotic control*

$$\text{Amount of antibiotic recovered, \%} = \frac{\text{(Antibiotic assayed, mg/mL)(Dilution factor, mL)}}{\text{(Antibiotic sample wt, mg)}} \times 100\%$$

3. *Calculations for indirect in vitro release studies.*

$$\text{Amount released, wt \% antibiotic} = \frac{\text{(Original core loading, wt \%) - (Final core loading, wt \%)}}{\text{(Original core loading, wt \%)}} \times 100\%$$

4. *Calculations for direct in vitro release studies.*

The receiving fluid (RF) is replaced after each sampling. The following equation is used to determine the cumulative amount of antibiotic released.

$$\text{Antibiotic released, mg} = \text{Antibiotic conc., mg/mL} \times \text{RF volume, mL} + \sum_{n-1}^{n} \left(\text{Antibiotic conc., mg/mL}\right) \times \left(\text{RF volume, mL}\right)$$

The following equation is used to calculate the percent of antibiotic released from the microspheres.

$$\text{Antibiotic released, \%} = \frac{\text{(Antibiotic released, mg)}}{\text{(Sample wt., mg) (Core loading/100)}} \times 100\%$$

D. Examination of Surface Morphology

It is important that microspheres have smooth surfaces with a continuous polymeric coating because pinholes or surface cracks would allow the drug to leach out of the microspheres prematurely. Therefore, we routinely examine the surface morphology of microspheres by scanning electron microscopy (SEM). A representative sample of microspheres is mounted on an aluminum SEM stub. The mounted sample is plasma cross-linked for 15 min using an ETEC Model Autoscan SEM (Haywood, CA) to rigidify the adhesive. The cross-linked sample mount is then sputter coated with a 60:40 Au/Pd alloy using a Hummer V Sputter Coater (Antech; Alexander, VA). The entire field of microspheres is examined and SEM photomicrographs are taken of a representative area. Magnifications can be chosen to yield one image of an area large enough to give a good representation of the whole batch of microspheres, one image having about 20 particles along each side of the photomicrograph, one image showing the microstructure of the surface of a typical microsphere, and one image of a cross section of a typical microsphere.

E. Determination of Particle-Size Distribution of Microspheres

Microsphere size has a large impact on release characteristics. That is, smaller microspheres release drug more rapidly than larger microspheres because small microspheres have larger surface area to mass ratios. Therefore, when we isolate a particular size fraction of microspheres, e.g. 45- to 425-μm microspheres, it is important to know how the microspheres are distributed within this size fraction. For example, the microspheres maybe evenly distributed within this fraction or skewed toward one end of the selected range.

Therefore, we routinely determine the size distribution of microsphere batches. A representative sample of the microspheres is suspended in an appropriate nonsolvent and placed in a sample cell. The cell is positioned in a Malvern Laser Diffraction Particle Sizer (Malvern Instruments; Malvern, England). A low-power visible laser transmitter produces a parallel, monochromatic beam of light that illuminates the microspheres. The incident light is diffracted by the illuminated microspheres to give a stationary diffraction pattern. A Fourier-transform lens focuses the diffraction pattern onto a multielement photoelectric detector which produces as analogue signal proportional to the incident light intensity. This detector is directly interfaced to a computer which reads the diffraction pattern and performs the necessary computations to generate a particle-size distribution.

IV. REFERENCES

1. Bawdon, R.E.; Leveno, K.J.; Quirk, J.G.; Cunningham, F.G.; Guss, S.P. High pressure liquid chromatographic assay of cefamandole in serum following intravenous and intraperitoneal administration. J. Liq. Chrom. 6:2747-2759; 1983.

2. Das Gupta, V.; Stewart, K.R.; Gunter, J.M. Quantitation of amikacin, kanamycin, neomycin and tobramycin in pharmaceutical dosage forms using the Hantzsch reaction. J. Am. Pharm. Sci. 72: 1470-1471; 1983.

3. Csiba, A. Spectrofluorimetric method for aminoglycoside antibiotics. J. Pharm. Pharmacol. 31:115-116; 1979.

APPENDIX A

Sample Transfer Forms

SAMPLE TRANSFER FORM

Sample Description:	Tobramycin microspheres
Composite Number:	H474-125-01S
Tobramycin Content:	9.1 wt %
Mean Particle Size:	357 μm
Sample Amount:	50 ± 1 g (5 X 10 g/vial)
Active Ingredient:	Tobramycin sulfate
Batch Number:	900606 (ICC Industries, Inc.; New York, NY)
Potency:	639.9 μg/mg
Excipient:	60:40 DL/PLG
Lot Number:	BPI 112-95-1 (Birmingham Polymer, Inc.; Birmingham, AL)
Inherent Viscosity:	0.5 dL/g
Solvent:	HFIP

COMMENTS:

(1) Store desiccated at 4 °C.

(2) Shake vials to break up microspheres before administering.

(3) Characterization data are given on pages 2 and 3 of this Sample Transfer Form. (Note: All core-loading and in vitro data are expressed in terms of tobramycin.)

(4) Sterilized with 2.5 (± 10%) Mrads of gamma radiation.

HAZARD UNKNOWN **NOT FOR USE IN HUMANS**

Released by:

David W. Mason
Head, Drug Delivery Section

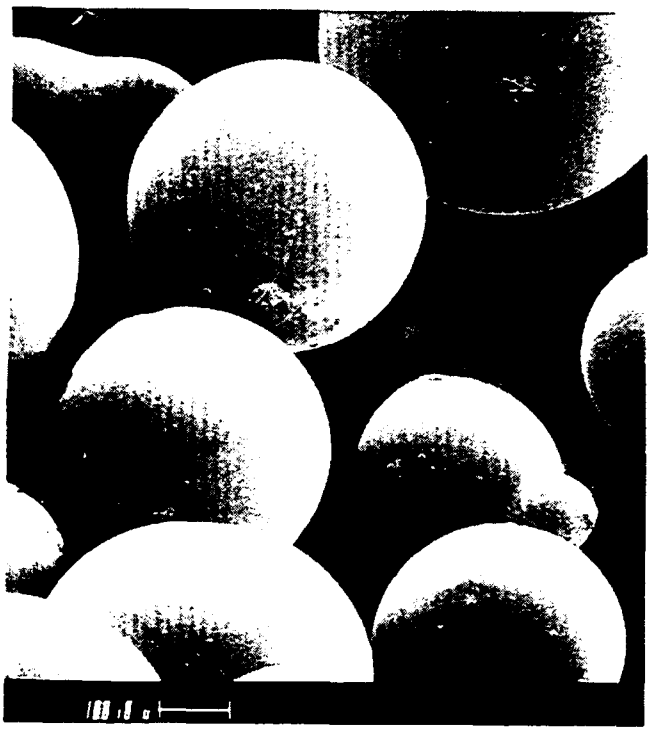
Teresa M. Ferrell
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

X183

H474-125-01 50X



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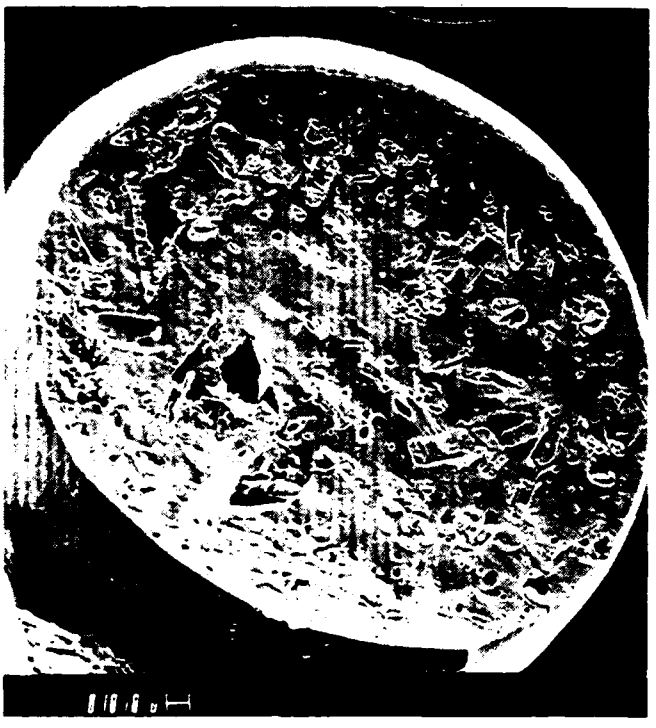

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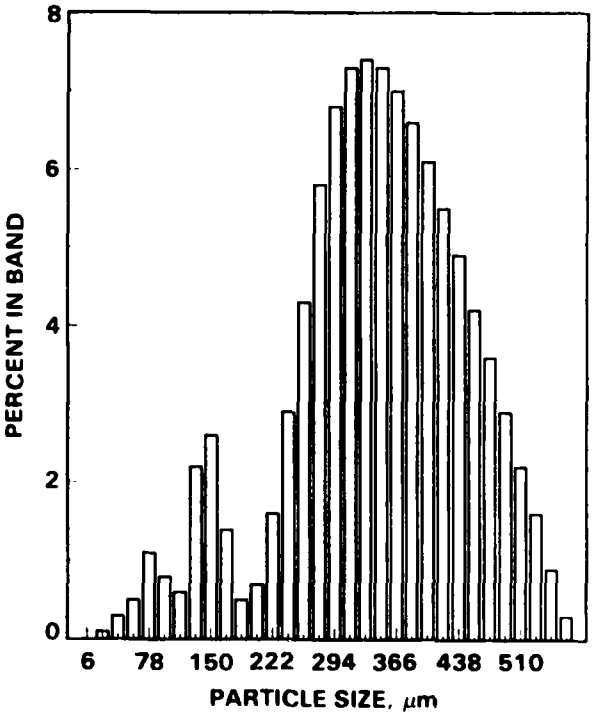
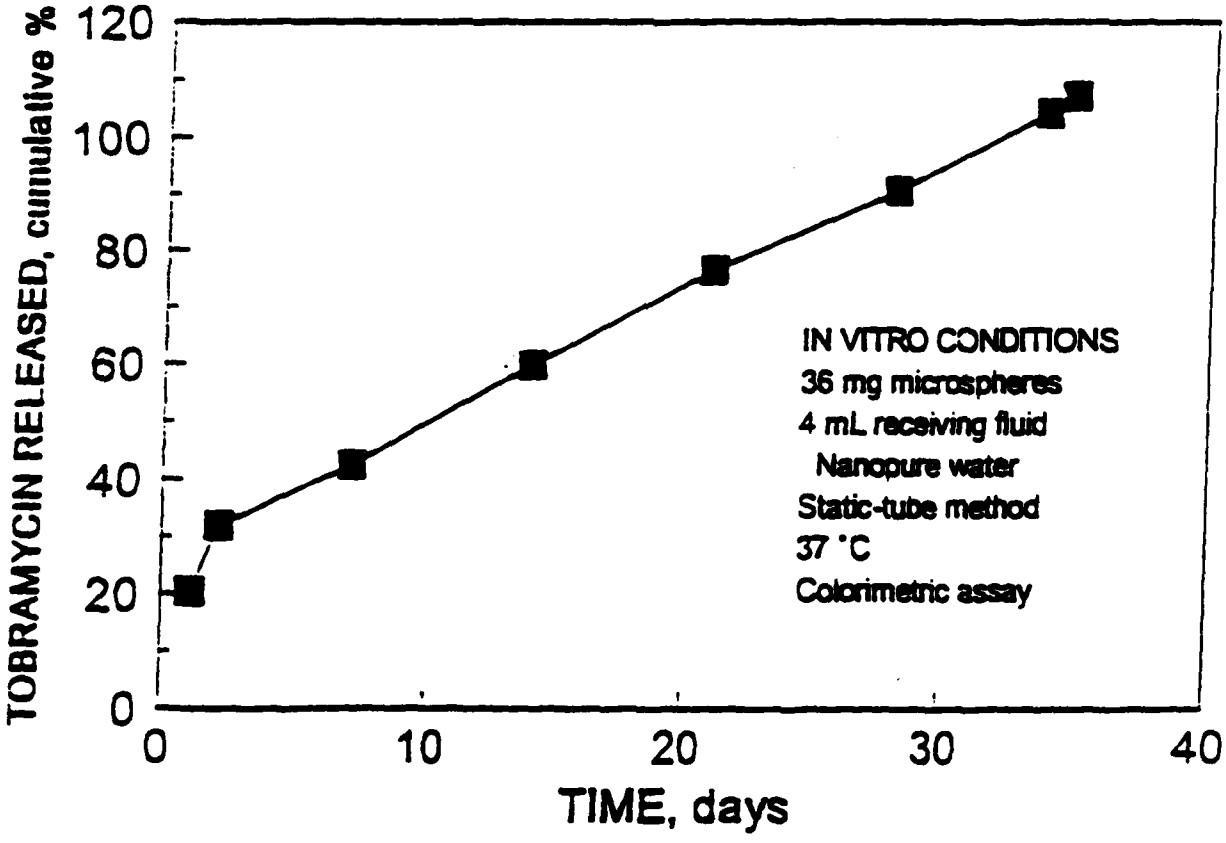


SEM photomicrographs and particle-size distribution of 9.1 wt.% tobramycin microspheres: Composite H474-125-01.

TOBRAMYCIN MICROSPHERES

COMPOSITE H474-125-01S



SAMPLE TRANSFER FORM

Sample Description:	Placebo microspheres for tobramycin study
Composite Number:	H474-149-01S
Mean Particle Size:	338 μm
Sample Amount:	25 ± 1 g (2 X 10 g/vial; 1 X 5 g/vial)
Excipient:	60:40 DL/PLG
Lot Number:	BPI 112-95-1 (Birmingham Polymer, Inc.; Birmingham, AL)
Inherent Viscosity:	0.5 dL/g
Solvent:	HFIP

COMMENTS:

(1) Store desiccated at 4 °C.

(2) Shake vials to break up microspheres before administering.

(3) Characterization data are given on page 2 of this Sample Transfer Form.

(4) Sterilized with 2.5 (± 10%) Mrad of gamma radiation.

HAZARD UNKNOWN **NOT FOR USE IN HUMANS**

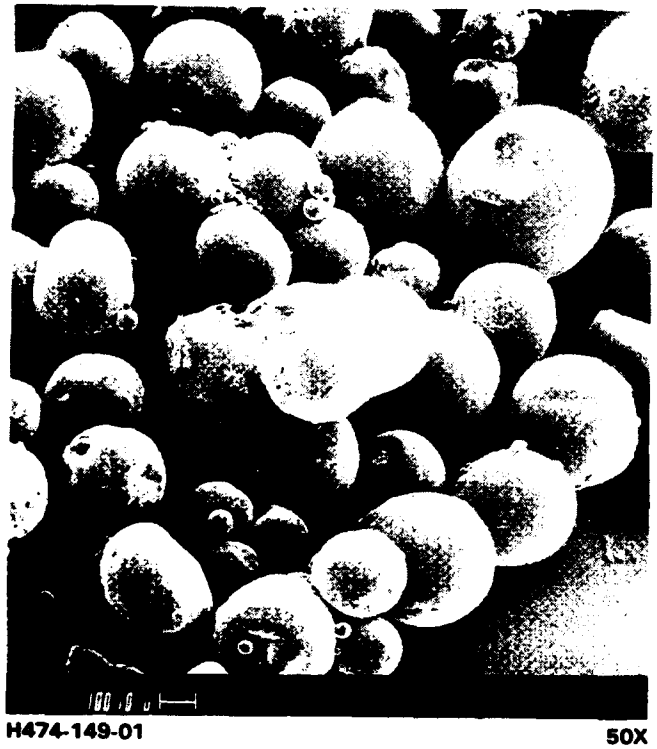
Released by:

David W. Mason
Head, Drug Delivery Section

Teresa M. Ferrell
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research



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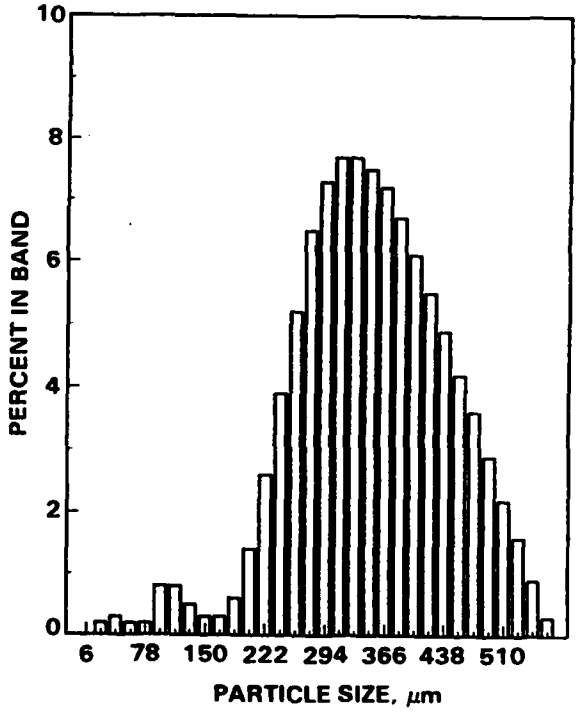


615-284

SEM photomicrographs and particle-size distribution of placebo microspheres: Composite H474-149-01 (placebo microspheres for tobramycin study).

SAMPLE TRANSFER FORM

Sample Description:	Tobramycin sulfate
Sample Number:	H326-099-001
Tobramycin Content:	65.6 wt%
Sample Amount:	25 ± 1 g
Batch Number:	900606 (ICC Industries, Inc.; New York, NY)
Potency:	639.9 μg/mg

COMMENTS: (1) Certificate of analysis attached.

(2) Shipped as received from the manufacturer.

HAZARD UNKNOWN **NOT FOR USE IN HUMANS**

Released by:

David W. Mason
Head, Drug Delivery Section

Teresa M. Ferrell
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

ICC INDUSTRIES INC.
PHARMACEUTICAL DIVISION
720 FIFTH AVENUE
NEW YORK, N.Y. 10019

CERTIFICATE OF ANALYSIS

PRODUCT:	TOBRAMYCIN SULFATE - NON STERILE
BATCH NO:	900606
QUANTITY:	500 GMS
ANALYSIS RESULT:	
MOISTURE:	1.94%
IDENTIFICATON:	POSITIVE
PH:	7.6
RESIDUE ON IGNITION:	0.49%
HEAVY METAL:	< 30 PPM
POTENCY:	639.9μ/MG
CONCLUSION:	CONFORMS TO USP22
MANUFACTURED BY:	HAIMEN PHARMACEUTICAL FACTORY JIAOJIANG CITY, ZHEJIANG PROVINCE PEOPLES REPUBLIC OF CHINA

212-903-1700 • CABLES. ICCTRADENEWYORK • TELEX: CCI 7607944 ITT 420778 • FAX 212-903-1726, 212-903-1794

SAMPLE TRANSFER FORM

Sample Description:	Cefamandole microspheres
Composite Number:	H326-093-01S
Cefamandole Content:	9.45 wt %
Mean Particle Size:	357 μm
Sample Amount:	50 ± 1 g (5 X 10 g/vial)
Active Ingredient:	Cefamandole, free acid
Batch Number:	440103-022-3 (Interchem Corp.; Paramus, NJ)
Potency:	793 μg/mg
Excipient:	60:40 DL/PLG
Lot Number:	BPI 112-95-1 (Birmingham Polymer, Inc.; Birmingham, AL)
Inherent Viscosity:	0.5 dL/g
Solvent:	HFIP

COMMENTS:

(1) Store desiccated at 4 °C.

(2) Shake vials to break up microspheres before administering.

(3) Characterization data are given on pages 2 and 3 of this Sample Transfer Form. (Note: All core-loading and in vitro release data are expressed in terms of cefamandole.)

(4) Sterilized with 2.5 (± 10%) Mrad of gamma radiation.

HAZARD UNKNOWN

NOT FOR USE IN HUMANS

Released by:

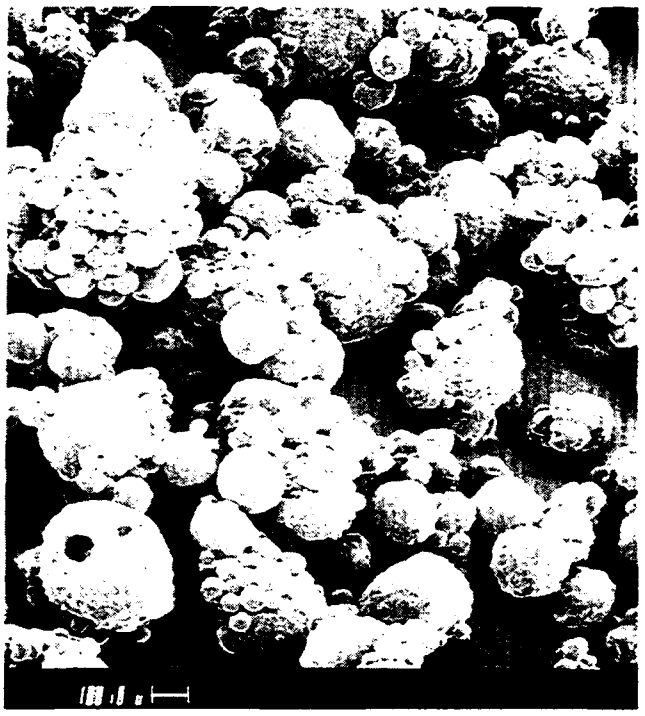
David W. Mason
Head, Drug Delivery Section

Teresa M. Ferrell
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

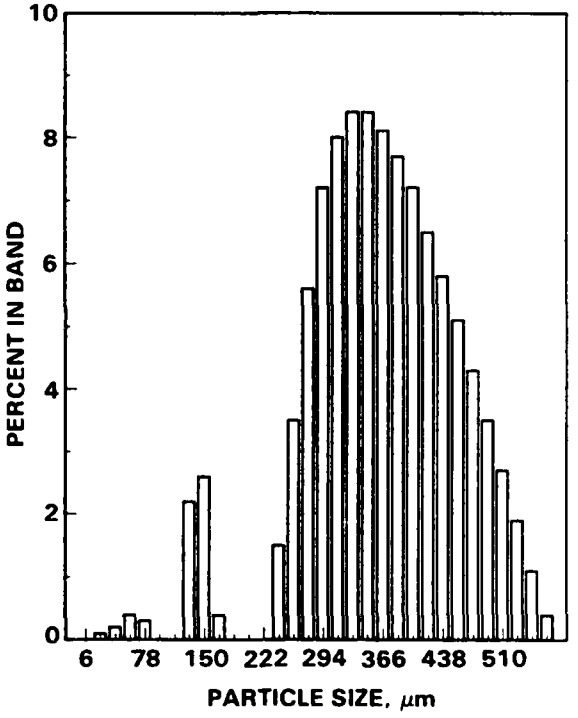
X183



H326-093-01 50X

H326-093-01 500X

H326-093-01 150X

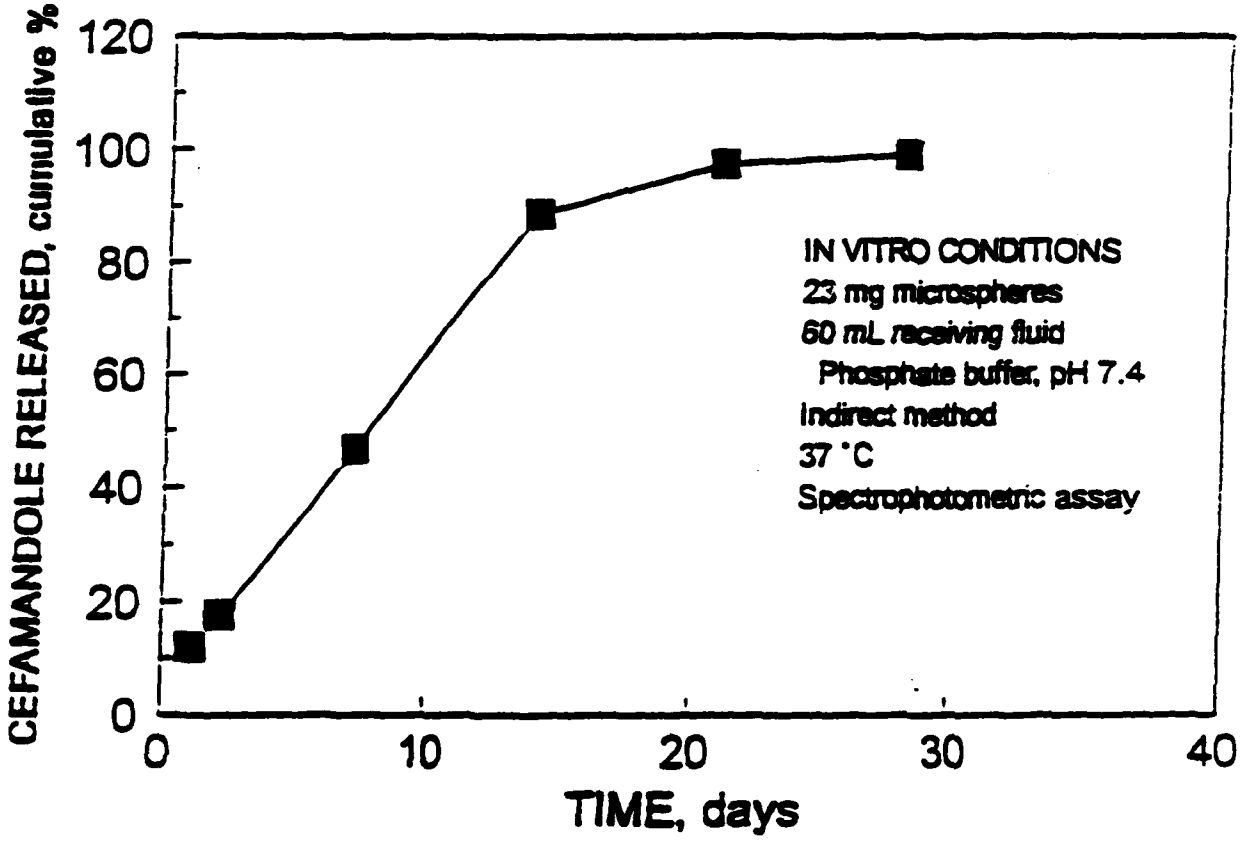


615-282

SEM photomicrographs and particle-size distribution of 9.5 wt.% cefamandole microspheres: Composite H326-093-01.

CEFAMANDOLE MICROSPHERES

COMPOSITE H326-093-01S



SAMPLE TRANSFER FORM

Sample Description:	Placebo microspheres for cefamandole study
Composite Number:	H326-096-01S
Mean Particle Size:	338 μm
Sample Amount:	35 ± 1 g (3 X 10 g/vial; 1 X 5 g/vial)
Excipient:	60:40 DL/PLG
Lot Number:	BPI 112-88-1 (Birmingham Polymer, Inc.; Birmingham, AL)
Inherent Viscosity:	0.51 dL/g
Solvent:	HFIP

COMMENTS:

(1) Store desiccated at 4 °C.

(2) Shake vials to break up microspheres before administering.

(3) Characterization data are given on page 2 of this Sample Release Form.

(4) Sterilized with 2.5 (± 10%) Mrad of gamma radiation.

HAZARD UNKNOWN

NOT FOR USE IN HUMANS

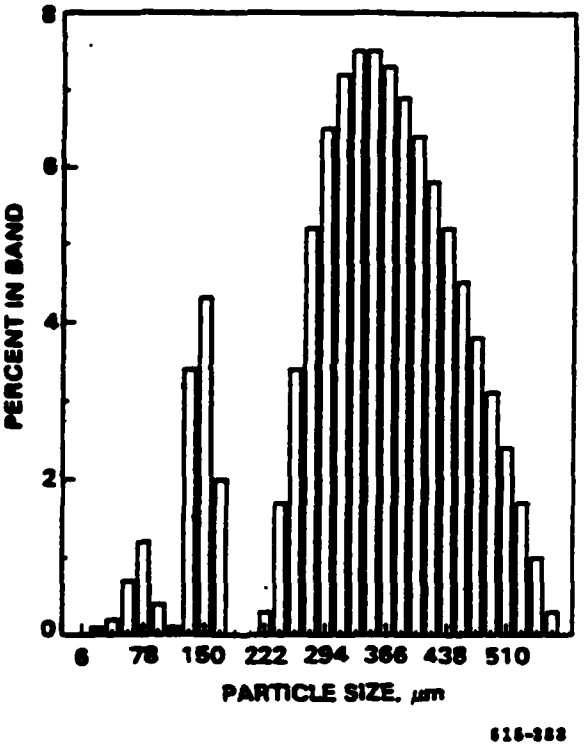
Released by:

David W. Mason
Head, Drug Delivery Section

Teresa M. Ferrell
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research



SEM photomicrographs and particle-size distribution of placebo microspheres: Composite H326-098-01 (placebo microspheres for cefamandole study).

SAMPLE TRANSFER FORM

Sample Description:	Cefamandole, free acid
Sample Number:	H326-099-002
Drug Content:	100 wt%
Sample Amount:	25 ± 1 g
Batch Number:	440103-022-3 (Interchem Corp.; Paramus, NJ)
Potency:	793 μg/mg

COMMENTS: (1) Certificate of Analysis attached.

(2) Shipped as received from the manufacturer.

HAZARD UNKNOWN **NOT FOR USE IN HUMANS**

Released by:

David W. Mason
Head, Drug Delivery Section

Teresa M. Ferrell
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

FEB [illegible] [illegible] P.[illegible]

ACS DOBFAR

[illegible]

Rif. cliente (Nv.ad) 02.07.93

INTERCHEM CORPORATION 120 ROUTE

17 NORTH SUITE 115 PARAMUS NJ

07652 USA

CERTIFICATE OF ANALYSIS

Product	CEFAMANDOLE FREE ACID			Date of manufacture	1.93
Batch N.	440103 002 3	Net weight	KG 0.5	Expiration date	1.95
Analysis record N.	PF0064	N. of packages	1	According to	LILLY SPECIFICATION

Appearance: WHITE TO OFF-WHITE GRANULAR POWDER

	Test	Results	Specifications	Units
Chemical and Physical	IDENTIFICATION	POSITIVE	POSITIVE	
	ASSAY	793	>=780	mcg/mg
	WATER	0.2	<=0.5	%
	CEFAMANDOLE IMPURITY	0.3	<=2.5	%
	O-ACETYL CEFAMANDOLE	0.0	<=1.0	%
	O-FORMYL MANDELOYL 7-ACA	0.2	<=1.0	%
	TETRAZOLE THIOL	<0.2	<=0.2	%
	COLOR (10% Acetone-475 nm)	0.006	<=0.075	Absorbance unit
	ACETONITRILE	12.8	<=15.0	%
	ETHYL ACETATE	0.0	<=0.5	%
	ETHYLENE DICHLORIDE	0.0	<=0.4	%
	METHYLENE CHLORIDE	0.0	<=0.5	%
Microbiological				
Biological				

This drug substance was manufactured in conformity with G.M.P. as recommended by WHO

PAGE 1

Notes	Issue date	The Head of Q.C. department
	JAN. 8 1993	DR. S. FAPANNI [signature]

COMMENTS:

(1) Store desiccated at 4 °C.

(2) Characterization data are given on pages 3 and 4 of this Sample Transfer Form. (Note: All core-loading and in vitro release data are expressed in terms of cefamandole.)

(3) Sterilized with 2.5 (± 10%) Mrad of gamma radiation at dry-ice temperature.

(4) Vial Numbers: H326-128-01 through H326-128-05.

H326-123-01S SURFACE 20X

H326-123-01S SURFACE 100X

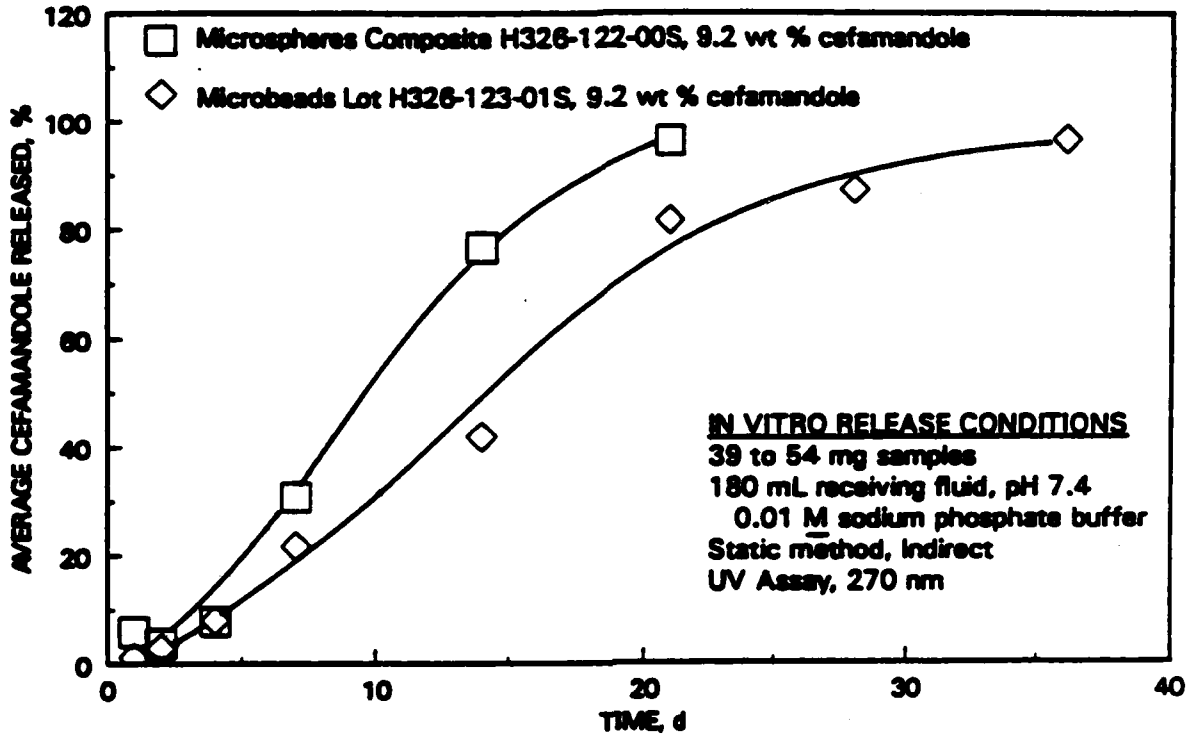
H326-123-01S LONGITUDINAL CROSS SECTION 20X

H326-123-01S RADIAL CROSS SECTION 20X

SEM photomicrographs of microbeads Lot H326-123-01S made from microsphere Composite H326-122-00.

CEFAMANDOLE RELEASE PROFILES

Microspheres and Microbeads



CEFAMANDOLE MICROSPHERES

Cefamandole Released, %

	Day 1	Day 2	Day 4	Day 7	Day 14	Day 21
	8.3	4.2	8.0	33.6	80.5	97.1
	3.4	3.7	7.6	30.3	74.8	96.0
	0.0	3.3	7.4	28.3	74.3	95.9
Average % released						

CEFAMANDOLE MICROBEADS

Cefamandole Released, %

	Day 1	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28	Day 36
	3.6	3.7	12.3	26.1	44.5	87.7	93.6	97.0
	0.0	3.5	6.9	20.9	44.3	81.5	91.2	95.5
	0.0	1.6	4.4	17.8	36.5	75.4	76.0	N/A
Average % released								

N/A = analyzed in duplicate.

SAMPLE TRANSFER FORM

Sample Description:	**Placebo Microbeads for Cefamandole Study**
Lot Number:	H326-127-01S
Drug Content:	0 wt % cefamandole
Bead Weight:	46 (± 10 %) mg
Bead Dimensions:	3.9 x 4.4 mm (± 5 %)
Sample Amount:	25 ± 1 g (2 X 10 g/vial; 1 X 5 g/vial)
Microsphere Component:	**Composite H326-126-01**
Drug Content:	0 wt % cefamandole
Microsphere Size Range:	10 to 1000 μm
Excipient:	**60:40 DL-PLG**
Source:	Birmingham Polymer, Inc.; Birmingham, AL
Lot Number:	BPI 112-95-1
Inherent Viscosity:	0.5 dL/g; as determined in hexafluoroisopropanol at 30 °C

COMMENTS:

(1) Store desiccated at 4 °C.

(2) Characterization data are given on page 2 of this Sample Transfer Form.

(3) Sterilized with 2.5 (± 10 %) Mrad of gamma radiation at dry ice temperature.

(4) Vial Numbers: H326-128-06 through H326-128-08.

HAZARD UNKNOWN **NOT FOR USE IN HUMANS**

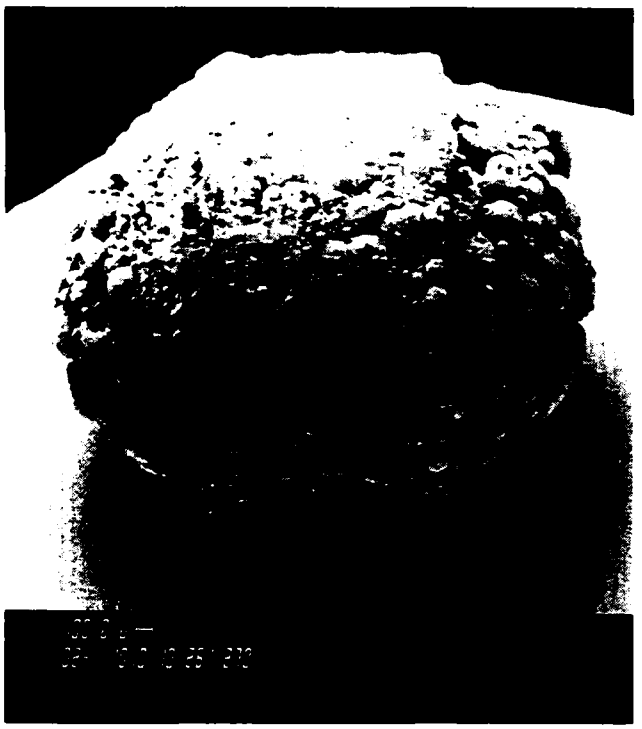
Released by:

David W. Mason
Research Engineer

Darryl F. Love
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research



H326-127-01S SURFACE 20X

H326-127-01S SURFACE 100X

H326-127-01S LONGITUDINAL CROSS SECTION 20X

H326-127-01S RADIAL CROSS SECTION 100X

SEM photomicrographs of placebo microbeads Lot H326-127-01S made from microsphere Composite H326-126-00.

SAMPLE TRANSFER FORM

Sample Description:	Cefamandole, Free Acid
Source:	Interchem Corp.; Pharamus, NJ
Batch Number:	440103-022-3
Drug Content:	As received
Sample Amount:	359 ± 1 g (1 x 50 g/container; 3 x 103 g/container)

COMMENTS:

(1) Certificate of Analysis attached.

(2) Store desiccated at room temperature.

(3) Contract deliverable (50 g) is in Container H326-130-01.

(4) All remaining cefamandole, free acid that was not part of the original contract deliverables is supplied in Containers H326-130-02 *through* H326-130-04.

HAZARD UNKNOWN **NOT FOR USE IN HUMANS**

Released by:

David W. Mason
Research Engineer

Darryl F. Love
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

X183

FEB 18 '93 11:03AM [illegible]

ACS DOBFAR

Rif. cliente [illegible] 02.02.93

INTERCHEM CORPORATION 120 ROUTE

17 NORTH SUITE 115 PARAMUS NJ

07652 USA

CERTIFICATE OF ANALYSIS

Product	CEFAMANDOLE FREE ACID			Date of manufacture	1.93
Batch n.	340103 002 3	[illegible]	KG 0.5	Expiration date	1.95
[illegible]	PF0364	[illegible]	1	According to	LILLY SPECIFICATION

Appearance: WHITE TO OFF-WHITE GRANULAR POWDER

Test	Results	Requirements	Units
IDENTIFICATION	POSITIVE	POSITIVE	
ASSAY	793	>=780	mcg/mg
WATER	0.2	<=0.5	%
CEFAMANDOLE IMPURITY	0.2	<=2.5	%
O-ACETYL CEFAMANDOLE	0.0	<=1.0	%
O-FORMYL MANDELOYL 7-ACA	0.2	<=1.0	%
TETRAZOLE THIOL	<0.2	<=0.2	%
COLOR (10% Acetone-475 nm)	0.006	<=0.075	Absorbance unit
ACETONITRILE	12.8	<=15.0	%
ETHYL ACETATE	0.0	<=0.5	%
ETHYLENE DICHLORIDE	0.0	<=0.4	%
METHYLENE CHLORIDE	0.0	<=0.5	%

PAGE 1

	JAN. 9 1993	DR. S. FARANNI

SAMPLE TRANSFER FORM

Sample Description:	**Cefamandole Nafate**
Source:	Interchem Corp.; Pharamus, NJ
Batch Number:	440105-037-2
Drug Content:	As received
Sample Amount:	486 ± 5 g (4 x 99 g/container; 1 x 90 g/container)

COMMENTS:

(1) Certificate of Analysis attached.

(2) Store desiccated at room temperature.

(3) Container Numbers: H326-131-01 through H326-131-05.

HAZARD UNKNOWN

NOT FOR USE IN HUMANS

Released by:

David W. Mason
Research Engineer

Darryl F. Love
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

FEB 15 '93 11:10AM INTERCHEM CORP P.3

Rif. cliente INV.60 02.02.93

INTERCHEM CORPORATION 120 ROUTE

17 NORTH SUITE 115 PARAMUS NJ

07652 USA

CERTIFICATE OF ANALYSIS

Product	CEFAMANDOLO NAFATE STERILE FOR INJ.			Date of manufacture	6.92
Batch N.	9401C5 037 2	Net weight	KG 0.5	Expiration date	6.94
Analysis request N	PF3687	N. of packages	035X	According to	USP
Appearance	WHITE CRYSTALLINE POWDER				

	Test	Results	Specifications	Units
Chemical and physical	IDENTIFICATION TLC	POSITIVE	POSITIVE	-
	pH (sol 100mg/ml)	6.8	6.0 TO 8.0	pH UNITS
	WATER	1.7	<=3.0	%
	POTENCY	861	810 TO 1000	mcg/mg (as CEFAMANDOLE) a
	SODIUM CARBONATE CONTENT	70.9	54.0 TO 72.0	mg/g of potency
	CONSTITUTED SOLUTION	COMPLIES	COMPLIES	
	FOREIGN PARTICLES			PARTICLES/c
	_PARTICLES 1 > 10.0 micron	44	<=400	PARTICLES/g
	_PARTICLES 5 > 25.0 micron	1	<=40	PARTICLES/g
Microbiological	STERILITY	COMPLIES	COMPLIES	-
Biological	BACTERIAL ENDOTOXINS (LAL Test)	<0.15	<=0.15	USP EU/mg CEFAMANDOLE NAFATE

The drug substance was manufactured in conformity with G.M.P. as recommended by WHO

PAGE 1

a=CALCULATED ON THE ANHYDROUS BASIS
b=CORRECTED FOR SODIUM CARBONATE

Release date: JUNE 26 1992

The head of Q.C. department: DR. S. PAPANI

SAMPLE TRANSFER FORM

Sample Description:	Tobramycin
Source:	ICC Industries, Inc.; New York, NY
Manufacturer:	Haimen Pharmaceutical Factory; Jiaojiang City, Peoples Republic of China
Batch Number:	921103
Drug Content:	As received
Sample Amount:	400 ± 50 g

COMMENTS:

(1) Certificate of Analysis attached.

(2) Store desiccated at 4 °C.

(3) Container Number: H326-052-01.

HAZARD UNKNOWN

NOT FOR USE IN HUMANS

Released by:

David W. Mason
Research Engineer

Darryl F. Love
Research Chemist

Received by:

Dr. Elliot Jacobs
U.S. Army Institute of
Dental Research

浙江海门制药厂

HAIMEN PHARMACEUTICAL FACTORY

CERTIFICATE OF ANALYSIS 检验报告单

TOBRAMYCINI 妥布拉霉素

BATCH 批号 NO.F.C 921103 MNF DATE 检验期 92.12.3 EXP DATE 有效期 96.11

SPECIFICATION 规格 [illegible] QUANTITY 数量 [illegible]

SOURCE 来源 [illegible] INVOICE NO 化验单号 079 DATE 报告日期 92.12.5

DESCRIPTION 性状 A white crystalline powder 白色结晶性粉末

TESTS 试验	RESULTS 结果	LIMITS 限度
IDENTIFICATION 鉴别	阳性 positive	POSITIVE 阳性
PH	9.8	9.0 ~ 11.0
WATER 水分	1.94%	≤8.0%
RESIDUE ON IGNITION 炽灼残渣	0.3%	≤1%
HEAVY METALS 重金属	少于30ppm	<30PPM
PYROGEN TEST 热源	合格 pass	—
ASSAY 效价 (生物测定)	930.6 ug/mg	≥900 ug/mg
HpLC 测定	919.9 ug/mg	

杂质 Impurity 1.0%

CONCLUSION 结论	The specifications conform with usp(22) 本品符合usp(22)
REMARKS 备注	

Q.C.DIRECTOR 质检科长 [signature stamp] COLLATOR 复核人 [signature stamp] ANALYST 化验员 [signature stamp]

SAMPLE TRANSFER FORM

Sample Description:	Tobramycin Sulfate
Source:	ICC Industries, Inc.; New York, NY
Manufacturer:	Haimen Pharmaceutical Factory; Jiaojiang City, Peoples Republic of China
Batch Number:	900606
Drug Content:	As received
Sample Amount:	400 ± 50 g

COMMENTS:

(1) Certificate of Analysis attached.

(2) Store desiccated at 4 °C.

(3) Container Number: H326-052-02.

HAZARD UNKNOWN

NOT FOR USE IN HUMANS

Released by:

David W. Mason
Research Engineer

Darryl F. Love
Research Chemist

Received by:

Dr. Elliot Jacob
U.S. Army Institute of
Dental Research

X183

ICC INDUSTRIES INC.
PHARMACEUTICAL DIVISION
720 FIFTH AVENUE
NEW YORK, N.Y. 10019

CERTIFICATE OF ANALYSIS

PRODUCT:	TOBRAMYCIN SULFATE - NON STERILE
BATCH NO:	900606
QUANTITY:	500 GMS
ANALYSIS RESULT:	
MOISTURE:	1.94%
IDENTIFICATON:	POSITIVE
PH:	7.6
RESIDUE ON IGNITION:	0.49%
HEAVY METAL:	< 30 PPM
POTENCY:	639.9μ/MG
CONCLUSION:	CONFORMS TO USP22
MANUFACTURED BY:	HAIMEN PHARMACEUTICAL FACTORY JIAOJIANG CITY, ZHEJIANG PROVINCE PEOPLES REPUBLIC OF CHINA

APPENDIX B

Certificates of Analysis for Polymers

BPI
BIRMINGHAM POLYMERS, INC.

BIRMINGHAM POLYMERS INCORPORATED
CERTIFICATE OF ANALYSIS

POLYMER

Polymer lot number	112-88-1
Polymer type	60/40 Poly(DL-lactide-co-glycolide)(nominal)
Monomer ratio, ^{1}H-NMR	59/41 lactide/glycolide
Inherent viscosity, dL/g	0.51
Viscometer type/no.	Cannon-Fenske A65/50
Solvent	HFIP
Concentration, g/dL	~0.5
Temperature, °C	30
Residual Sn^{+2}	32.5
Bioburden	See attachments

MONOMERS

Type	Lot Number
Dl-lactide	110-49-1
Glycolide	110-85-1

By: [signature] 4/5/97

Warranty (Limitation of Liability) - BPI warrants that its products are free from defects in workmanship and materials and are made in accordance with applicable specifications and Current Good Manufacturing Practices of the FDA. BPI MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, AND DISCLAIMS ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR PURPOSE.

Specializing in Biodegradable Polymers
110 40th Street North Birmingham, Alabama 35222 205-595-2231 Fax 205-595-2240

Bioburden Determination

Linvatec
Concept Arthroscopy
11311 Concept Boulevard
Largo, Florida 34643
813 392-6464
Fax 813 399-2628

Per QAP - 215

Lab Number: 93-8198
Date Of Test: 2-23-93
Date Completed: 2-26-93
Material Tested: Raw Polymer

Sample Number	Aerobic CFU's/Sample	Aerobic Spores CFU's/Sample	Anaerobic CFU's/Sample
112-78-1	0	0	0
[redacted]			
112-95-2	0	0	0
115-14-1	0	0	0
115-15-1	0	0	0
115-16-1	0	0	0
115-17-1	0	0	0

Total Average Aerobic CFU's: 0
Environmental Room Control Aerobic: Negative
Negative Control Of BF: Negative
Negative Control Of AA: Negative
Negative Control Of SCDA: Negative
Gas Pak Lot Number/Exp. Date: NA
Positive Control Of AA: Positive
Positive Control Of SCDA: Positive
Lot Number - AA: 93A-011901
Lot Number - SCDA: 93A-20203
BF Lot Number: 93A-012701
Anaerobic System Lot Number/Exp. Date: 2035009, Exp. 4/94
B. Subtilis Lot Number/Exp. Date: 010493, Exp. 1-4-94
C. Sporogenes Lot Number/Exp. Date: 090192, Exp. 9/93

Technician: Kimberly LaMere
Date: 3-3-93
Approved: Dennis Matt
Date: 3-10-93

Form 210-10

Rec. 03/25/93

LAL Assay For Endotoxin

Linvatec
Concept Arthroscopy

11311 Concept Boulevard
Largo, Florida 34643

813 392-6464
Fax 813 399-2600

Per QAP - 245 and USP XXII

Lab Number: 93-8232	Date: 3-8-93	Lead Number:
Material: Raw Polymer		Lot Number: 112-88-1
Part Number:		LAL Lot Number: 21-31-563
US RSE: EC-5	EU/Vial: 10,000	STD Conc. of Endpoint: 0
CSE Lot Number: 55	Potency of LAL: 0.25 Eu/ml	Potency of CSE: 10.0 Eu/ng
Extract Prepared: 3-8-93		Assayed: 3-8-93
Sterile H_2O Lot Numbers - Product Expiration/Exp. Date: J281277, Exp. 10/95		
Injection H_2O/Exp. Date: 57-197DK, Exp. 10/93	Dilutions H_2O: 93A-20203	

Standard Results

	CSE (EU/ML)				
	1.0	0.5	0.25	0.125	.0625
1	+	+	+	-	-
2	+	+	+	-	-

Sample

Gram:	Unit(s): 1.0 g	
Flushed:	Covered with: X	ml Pyrogen - free water: 10 mLs

Sample Results

Inhibition (Product) Control: +	Test Solution: -	H_2O: -

Comments

The results of this test indicate bacterial endotoxins: ○ WERE ⊘ WERE NOT present in the test solution at the level of detection employed.

Date Completed	Approved	Technician
3-16-93	Diane Yu	Kimberly Lamm

Form 249-9C

BPI

BIRMINGHAM POLYMERS, INC.

BIRMINGHAM POLYMERS INCORPORATED
CERTIFICATE OF ANALYSIS

Date February 2, 1993

POLYMER

Polymer lot no.	112-95-1
Polymer type	60/40 Poly(DL-lactide-co-glycolide) (nominal)
Monomer ratio, ^{1}H-NMR	59/41 lactide/glycolide
Inherent viscosity, dL/g	0.50
Viscometer type/no:	Cannon-Fenske A65 / 50
Solvent:	HFIP
Concentration, g/dL	~ 0.5
Temperature, °C:	30
Residual Sn^{+2}, ppm	34.1
Bioburden	See attachments

MONOMERS

Type	Lot No.
DL-lactide	110-76-3
Glycolide	110-66-2

By: _____

James P. English, P.E.

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Bioburden Determination

Linvatec
Concept Arthroscopy

11311 Concept Boulevard
Largo, Florida 34643

813 392-6464
Fax 813 399-2603

Per QAP - 215

Lab Number
92-7988

Date Of Test
12-15-92

Date Completed
12-18-92

Material Tested
Birmingham Polymers

Sample Number	Aerobic CFU's/Sample	Aerobic Spores CFU's/Sample	Anaerobic CFU's/Sample
112-94-1	0	0	0
112-95-1	0	0	0

Total Average Aerobic CFU's
0

Environmental Bench Control Results
Negative

Negative Control Of DF
Negative

Negative Control Of AA
Negative

Negative Control Of SCDA
Negative

Gas Pak Lot Number/Exp. Date
N/A

Positive Control Of AA
Positive

Positive Control Of SCDA
Positive

Lot Number - AA
92A-111002

Lot Number - SCDA
92A-111301

DF Lot Number
92A-120801

Anaerobic System Lot Number/Exp. Date
2035009, Exp. 4/94

B. Subtilis Lot Number/Exp. Date
110992, Exp. 11/9/92

C. Sporogenes Lot Number/Exp. Date
090192, Exp. 9/93

Technician
Kimberly LaMaster

Date
12-23-92

Approved
Dennis Martin

Date
12-23-92

Form 215-18

LAL Assay For Endotoxin

Linvatec
Concept Arthroscopy

11311 Concept Boulevard
Largo, Florida 34643

813 392-6464
Fax 813 399-2603

Per QAP - 245 and USP XXII

Lab Number: 92-7982	Date: 12-14-92	Load Number: N/A
Material: Raw Polymer		Lot Number: 112-95-1
Part Number: N/A		LAL Lot Number: 21-31-563
US RSE: EC-5	EU/Vial: 10,000	STD Dev. of Endpoint: 0
CSE Lot Number: 52	Potency of LAL: 0.25 Eu/ml	Potency of CSE: 10.0 Eu/ng
Extract Prepared: 12-8-92		Assayed: 12-8-92
Sterile H_2O Lot Numbers - Product Extractions/Exp. Date: J2N277, Exp. 10/95		
Injection H_2O/Exp. Date: 56-494DK, Exp. 9/93	Dilutions H_2O: 92A-102103	

Standard Results

	CSE (EU/ML)				
	1.0	0.5	0.25	0.125	.0625
1	+	+	+	-	-
2	+	+	+	-	-

Sample

Gram	Unit(s): 1.0 g	
Flushed	Covered with: X	ml Pyrogen - free water: 10 mls

Sample Results

Inhibition (Product) Control: +	Test Solution: -	H_2O: -

Comments

The results of this test indicate bacterial endotoxins: ○ WERE (X) WERE NOT present in the test solution at the level of detection employed.

Date Completed	Approval	Technician
12-23-92	Dennis Mast	Kimberly A. Masters

Form 245-1C

BPI

BIRMINGHAM POLYMERS, INC.

BIRMINGHAM POLYMERS INCORPORATED
CERTIFICATE OF ANALYSIS

Date October 16, 1992

POLYMER

Polymer lot no.	112-68-1
Polymer type	60/40 Poly(DL-lactide-co-glycolide) (nominal)
Monomer ratio, ^{1}H-NMR	60/40 lactide/glycolide
Inherent viscosity, dL/g	0.48
Viscometer type/no:	Cannon-Fenske A65 / 50
Solvent:	HFIP
Concentration, g/dL	~ 0.5
Temperature, °C:	30
Residual Sn^{+2}, ppm	57
Bioburden	See attachments

MONOMERS

Type	Lot No.
DL-lactide	110-40-1
Glycolide	110-51-1

By: _____

James P. English, P.E.

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LAL Assay For Endotoxin

Linvatec
Concept Arthroscopy

11311 Concept Boulevard
Largo, Florida 34643

813 392-6464
Fax 813 399-2603

Per QAP - 245 and USP XXII

Lab Number	Date	Load Number
92-7674	9-23-92	N/A

Material: RAW POLYMER
Lot Number: 112-68-1

Part Number: N/A
LAL Lot Number: 21-09-550

US RSE	EU/Vial	STD Dev. of Endpoint
EC-5	10,000	0

CSE Lot Number	Potency of LAL	Potency of CSE
53	0.25 Eu/ml	10.0 Eu/ng

Extract Prepared: 9-15-92
Assayed: 9-15-92

Sterile H_2O Lot Numbers - Product Extractions/Exp. Date: J2C312 exp. 3/95

Injection H_2O/Exp. Date: 57-197 DK exp. date: 10/93
Dilutions: D/P 92A-60905

Standard Results

	CSE (EU/ML)				
	1.0	0.5	0.25	0.125	.0625
1	+	+	+	-	-
2	+	+	+	-	-

Sample

Grams: 0.9
Units:

Flushed:
Covered with: X
ml Pyrogen - free water: 10

Sample Results

Inhibition (Product) Control: +

Test Solution: -

H_2O: -

Comments

The results of this test indicate bacterial endotoxins ○ WERE ⊗ WERE NOT present in the test solution at the level of detection employed.

Date Completed: 9-23-92
Approval: [signature]
Technician: [signature]

Form 245-10

Linvatec

11311 Concept Boulevard Largo, FL 34643 813 392-6464 Fax: 813 399-5255

BIOBURDEN DETERMINATION
(PER QAP-215)

LAB NUMBER: 92-7668
DATE OF TEST: 9-22-92
DATE COMPLETED: 9-25-92
MATERIAL TESTED: Birmingham Polymers
TOTAL RECOVERABLE.....

SAMPLE NUMBER	AEROBIC: CFU'S/SAMPLE	AEROBIC SPORES: CFU'S/SAMPLE	ANAEROBIC: CFU'S/SAMPLE
112-68-1 11	0	0	0
112-73-1 12	0	0	0
112-75-1 13	0	0	0
112-76-1 14	0	0	0

TOTAL AVERAGE AEROBIC CFU'S: 0
ENVIRONMENTAL BENCH CONTROL RESULTS: 0
NEGATIVE CONTROL OF DF: Negative
NEGATIVE CONTROL OF AA: Negative
NEGATIVE CONTROL OF SCDA: Negative
POSITIVE CONTROL OF AA: Positive
POSITIVE CONTROL OF SCDA: Positive
LOT NUMBER - AA: 92A-81902
LOT NUMBER - SCDA: 92A-91404
DF LOT NUMBER: 92A-90402
ANAEROBIC SYSTEM LOT NUMBER: 2025006, Exp. 3/94
GAS PAK LOT NUMBER: N/A
B. SUBTILIS LOT NUMBER/EXP. DATE: 082492, Exp. 8/93
C. SPOROGENES LOT NUMBER/EXP. DATE: 040192, Exp. 4/93

Kimberly LaMaster — 9-28-92
TECHNICIAN — DATE

Denise C. Sorensen — 9-28-92
APPROVAL — DATE

FORM 215-1A

APPENDIX C

Certificates of Analysis for Drugs

浙江海门制药厂

HAIMEN PHARMACEUTICAL FACTORY

CERTIFICATE OF ANALYSIS 检验报告单

TOBRAMYCINI 妥布拉霉素

BATCH 批号 NO.F.C 921103	MNF DATE 检验期 92.12.3	EXP DATE 有效期 96.11
SPECIFICATION 规格 妥布霉素	QUANTITY 数量 0.5Kg	
SOURCE 来源 妥布霉素车间	INVOICE NO 化验单号 079	DATE 报告日期 92.12.5
DESCRIPTION 性状	A white crystalline powder 白色结晶性粉末	

TESTS 试验	RESULTS 结果	LIMITS 限度
IDENTIFICATION 鉴别	阳性 positive	POSITIVE 阳性
PH	9.8	9.0 ~ 11.0
WATER 水份	1.94%	≤8.0%
RESIOUE ON IGNITION 炽灼残渣	0.3%	≤1%
HEAVY METALS 重金属	小于30ppm	<30PPM
PYROGEN TEST 热源	合格 pass	—
ASSAY 效价 (生物测定)	930.6 u/mg	≥900 u/mg
HPLC 测定	919.9 u/mg	
杂质 Impurity	1.0%	

CONCLUSION 结论	The specifications conform with usp(22) 本品符合usp(22)
REMARKS 公章	

Q.C.DIRECTOR 质检科长 [illegible] COLLATOR 复核人 [illegible] ANALYST 化验员 [illegible]

ICC INDUSTRIES INC.
PHARMACEUTICAL DIVISION
720 FIFTH AVENUE
NEW YORK, N.Y 10019

CERTIFICATE OF ANALYSIS

PRODUCT:	**TOBRAMYCIN SULFATE - NON STERILE**
BATCH NO:	**900606**
QUANTITY:	**500 GMS**
ANALYSIS RESULT:	
MOISTURE:	**1.94%**
IDENTIFICATON:	**POSITIVE**
PH:	**7.6**
RESIDUE ON IGNITION:	**0.49%**
HEAVY METAL:	**< 30 PPM**
POTENCY:	**639.9μ/MG**
CONCLUSION:	**CONFORMS TO USP22**
MANUFACTURED BY:	**HAIMEN PHARMACEUTICAL FACTORY JIAOJIANG CITY, ZHEJIANG PROVINCE PEOPLES REPUBLIC OF CHINA**

212-903-1700 • CABLES: ICCTRADENEWYORK • TELEX: CCI 7607944 ITT 420778 • FAX: 212-903-1726, 212-903-1794

Rif. cliente INV.60 02.02.93

ACS DOBFAR S.p.A.

[illegible] Legale e Amministrativa
[illegible] TRIBIANO (Milano) - Viale Addetta [illegible]
[illegible]
[illegible] TRIBIANO (MI)
[illegible] - Tel (02) 90693 1 - Fax (02) [illegible] - Telex [illegible] ACSITA I
[illegible] - Tel (02) [illegible] 1 - Fax (02) 9063 [illegible]
[illegible] - Tel (02) [illegible] 171 - 90631962
[illegible] VIMERCATE (MI)
[illegible] - Tel (039) 6081614 - Fax (039) 6081 [illegible] - Telex 351317 [illegible] - 334585 [illegible]

INTERCHEM CORPORATION 120 ROUTE
17 NORTH SUITE 115 PARAMUS NJ
07652 USA

CERTIFICATE OF ANALYSIS

Product	CEFAMANDOLE FREE ACID			Date of manufacture	1.93
Batch N.	440103 002 3	Net weight	KG 0.5	Expiration date	1.95
Analysis record N.	PF0064	N. of packages	X	According to	LILLY SPECIFICATION

Appearance WHITE TO OFF-WHITE GRANULAR POWDER

	Test	Results	Specifications	Units
Chemical and physical	IDENTIFICATION	POSITIVE	POSITIVE	
	ASSAY	793	>=780	mcg/mg
	WATER	0.2	<=0.5	%
	CEFAMANDOLE IMPURITY	0.3	<=2.5	%
	O-ACETYL CEFAMANDOLE	0.0	<=1.0	%
	O-FORMYL MANDELOYL 7-ACA	0.2	<=1.0	%
	TETRAZOLE THIOL	<0.2	<=0.2	%
	COLOR (10% Acetone-475 nm)	0.006	<=0.075	Absorbance unit
	ACETONITRILE	12.8	<=15.0	%
	ETHYL ACETATE	0.0	<=0.5	%
	ETHYLENE DICHLORIDE	0.0	<=0.4	%
	METHYLENE CHLORIDE	0.0	<=0.5	%
Microbiological				
Biological				

This drug substance was manufactured in conformity with G M P as recommended by WHO

PAGE 1

Notes	Assay date	The head of Q.C. department
	JAN. 8 1993	DR. S. FAPANNI

ACS DOBFAR S.p.A.

Sede Legale e Amministrativa
[illegible] (Milano) - Viale Addetta 4/6/10
Stabilimenti:
[illegible] (Mi):
[illegible] - Tel. (02) 90993 1 - Fax (02) 9064996 - Telex 340544 ACSITA I
[illegible] 9 - Tel. (02) 90983 1 - Fax (02) 90631158
[illegible] - Tel. (02) 90631171 - 90631983
[illegible] VIMERCATE (Mi)
[illegible] 7/9 - Tel. (039) 6081510 - Fax (039) 6081114 - Telex 351317 [illegible] - 324585 [illegible]

Rif. cliente INV.60 02.02.93

INTERCHEM CORPORATION 120 ROUTE
17 NORTH SUITE 115 PARAMUS NJ
07652 USA

CERTIFICATE OF ANALYSIS

Product:	CEFAMANDOLO NAFATE STERILE FOR INJ.			Date of manufacture	6.92
Batch N.	440105 037 2	Net weight	KG 0.5	Expiration date	6.94
Analysis record N	PF3687	N. of packages	035X	According to	USP

Appearance: WHITE CRYSTALLINE POWDER

	Test	Results	Specifications	Units
Chemical and physical	IDENTIFICATION TLC	POSITIVE	POSITIVE	-
	pH (sol 100mg/ml)	6.8	6.0 TO 8.0	pH UNITS
	WATER	1.7	<=3.0	%
	POTENCY	861	810 TO 1000	mcg/mg (as CEFAMANDOLE)$
	SODIUM CARBONATE CONTENT	70.9	54.0 TO 72.0	mg/g of potency
	CONSTITUTED SOLUTION	COMPLIES	COMPLIES	
	FOREIGN PARTICLES			PARTICLES/g
	_PARTICLES $ > 10.0 micron	44	<=400	PARTICLES/g
	_PARTICLES $ > 25.0 micron	1	<=40	PARTICLES/g
Microbiological	STERILITY	COMPLIES	COMPLIES	-
Biological	BACTERIAL ENDOTOXINS (LAL Test)	<0.15	<=0.15	USP EU/mg CEFAMANDOLE NAFATE

This drug substance was manufactured in conformity with G M P as recommended by WHO

PAGE 1

Notes	Assay date	The head of Q.C. department
$=CALCULATED ON THE ANHYDROUS BASIS $=CORRECTED FOR SODIUM CARBONATE	JUNE 26 1992	DR. S. FAPANNI

SUPPLEMENTARY

INFORMATION

DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
FORT DETRICK, FREDERICK, MD 21702-5012

REPLY TO ATTENTION OF:

ERRATA
AD-B189615

MCMR-RMI-S (70-1y) 3 Jan 97

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Contract Number DAMD17-92-C-2014. Request the limited distribution statement for Accession Document Number ADB183615 be changed to "Approved for public release; distribution unlimited." A copy of this report should be released to the National Technical Information Service.

2. Point of contact for this request is Mrs. Judy Pawlus at DSN 343-7322.

FOR THE COMMANDER:

for CORNELIUS R. FAY III
Lieutenant Colonel, MS
Deputy Chief of Staff for
Information Management

ERRATA